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Yeast Killer Toxin-Like Anti-Idiotypic Antibodies

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Anti-idiotypic antibodies (anti-Ids) were raised in a rabbit against a murine monoclonal antibody (MAB) neutralizing the yeast killer toxin produced by a strain of *Pichia (Hansenula) anomala*. In an immunodiffusion test, the anti-Ids produced in the rabbit recognized the antigen-binding site of the MAB used as the immunogen (KT4) but not that of another heterologous MAB. The absence of any significant cross-reactivity among the anti-Ids raised in a rabbit for a heterologous MAB suggested that the anti-Ids were highly specific for unique variable-region determinants. Furthermore, the *P. anomala* killer toxin proved to be competing with anti-Ids for the binding site of MAB KT4. Anti-Ids against the MAB to yeast killer toxin inhibited the growth of *Candida albicans*, thereby mimicking the effect of the yeast killer toxin. These results suggest that, in some cases, anti-Ids might be useful tools for elucidating structure-function relationships for sensitive cell receptors.

Basically, killer toxins are polypeptides secreted by killer yeasts of many genera which kill susceptible microorganisms. Cytoplasmatically inherited killer determinants have been identified as double-stranded RNA or DNA plasmids (21). Originally, the killer phenomenon was considered to be restricted to yeasts (2, 4, 10, 20).

With these perspectives, susceptibility to killer toxins of pathogenic yeasts has been extensively studied in our institute for epidemiological purposes (7, 11, 12), as well as for evaluation of their potential therapeutic effect (13). Recently, we observed that the yeast killer phenomenon was also displayed against a wide range of unrelated microorganisms (15), thus suggesting a possible unique form of bioaction. In yeasts, toxic action involves an initial binding of killer toxin to a cell wall receptor (1, 3).

Recently, we produced monoclonal antibodies (MABs) against the killer toxin of a selected yeast species (*Pichia [Hansenula] anomala* UCSC 25F) for use in a one-step purification of the killer toxin by affinity chromatography and the serological analysis of killer toxins produced by yeasts with different killer determinants (16).

In recent years, there have been several reports concerning the use of anti-idiotypic antibodies (anti-Ids) as probes for receptors (6, 8, 19). Furthermore, anti-Ids may mimic the original antigen by displaying some of its properties. Such procedures could represent a useful tool to better study the nature of toxin receptors on a sensitive cell surface.

The present study was initiated to produce anti-Ids in a rabbit against a MAB neutralizing a yeast killer toxin and to test their ability to simulate the properties of the yeast killer toxin itself.

Hybridoma cells. Hybridoma cells secreting MAB KT4 against yeast killer toxin were produced, selected, and cloned as previously described (16). Hybridoma cells producing a heterologous MAB (*Candida albicans* UCSC 1 [CA1]) were obtained from an additional fusion by previously described methods, as well as for the production of ascites fluids (14).

MAB purification. MABs KT4 and CA1 were partially purified with ascites fluids by precipitation with ammonium

sulfate (50% of saturation). The immunoglobulin fractions were dissolved and dialyzed three times against phosphate-buffered saline (pH 7.4). The immunoglobulin of MAB KT4 was detected by a double immunodiffusion procedure with *P. anomala* UCSC 25F killer toxin as the antigen. Protein concentrations (20 mg/ml) were determined by the Lowry method (5).

Fungal cultures and production of yeast killer toxin. Yeast killer strain *P. anomala* UCSC 25F, from which the crude killer toxin had been produced (16), and *C. albicans* CDC B385, a susceptible strain, were obtained from our culture collection.

Anti-Ids. Anti-Ids were raised in a New Zealand White rabbit by three intramuscular injections at weekly intervals of 5×10^6 hybridoma cells in incomplete Freund adjuvant (diluted 1:1). Before being tested, the rabbit antiserum was concentrated (5 \times) by B125 Minicon macrosolute concentrators (Amicon Corp., Lexington, Mass.).

Absorption procedures. Purified MAB KT4 was placed in 1-ml quantities in plastic tubes with 1 ml of *P. anomala* UCSC 25F crude killer toxin and allowed to stand overnight at 4°C. The mixture was then centrifuged at $12,063 \times g$ for 10 min. The supernatant was decanted, and the initial volume of the MAB was restored with Minicon concentrators.

Serological techniques. An agar-gel double immunodiffusion procedure was used in this study. Preparation of media and execution of the tests were carried out according to the recommendations of the Division of Mycotic Diseases, Centers for Disease Control, Atlanta, Ga., for the serological diagnosis of histoplasmosis (9). The slides were dried and stained by the Coomassie blue protein staining procedure.

Expression of anti-Id killer activity. The expression of the killer activity of anti-Ids was evaluated by a germ-agar-like immunodiffusion procedure. Briefly, a tube containing 10 ml of agarose (1%) in Tris barbiturate buffer (LKB-Produkter AB, Bromma, Sweden), used for the electrophoresis and diffusion technique, was melted, maintained at 45°C in a water bath, and seeded with a light suspension of *C. albicans* CDC B385, a yeast known to be susceptible to the action of the killer toxin of *P. anomala* UCSC 25F. The germ-agar medium was poured on a precoated glass slide for the immunodiffusion procedure and allowed to solidify at room temperature before the two wells (8 mm in diameter) were made. The first well was filled with the concentrated (5 \times),

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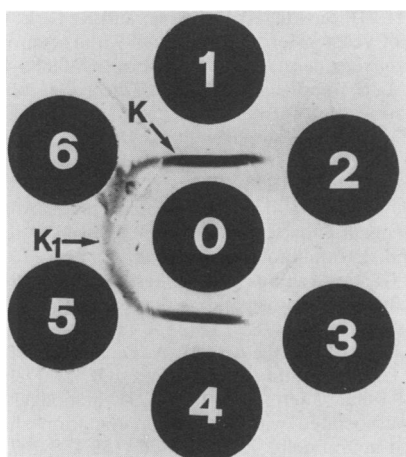


FIG. 1. Rabbit immunodiffusion pattern. Wells: 1 and 4, *P. anomala* UCSC 25F yeast killer toxin; 2 and 3, rabbit serum before immunization; 5 and 6, rabbit antiserum; 0, MAb KT4.

heat-inactivated (56°C for 30 min) normal rabbit serum collected before immunization, and the second well was filled with the concentrated (5×), heat-inactivated rabbit antiserum obtained after immunization. The slide was then incubated at 25°C for 72 h under high humidity. After this period, the slide was observed for evidence of growth around the wells. This procedure was repeated with a heterologous *Trichosporon capitatum* rabbit antiserum.

In the double immunodiffusion procedure, the concentrated rabbit serum reacting with MAb KT4 showed a precipitin band (K1). K1 proved to be homologous to the precipitin band (K) resulting from the reaction between *P. anomala* UCSC 25F yeast killer toxin and MAb KT4 (reference system). No reaction was observed between MAb KT4 and the concentrated normal rabbit serum, collected before immunization, used as a control (Fig. 1). No heterologous reaction was observed when the rabbit antiserum was tested with MAb CA1 (Fig. 2).

The absorption of MAb KT4 with *P. anomala* UCSC 25F yeast killer toxin caused the disappearance of the K1 pre-

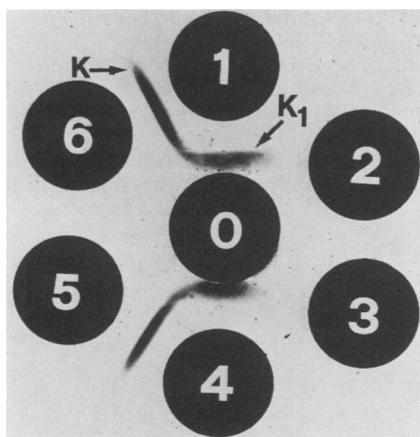


FIG. 2. Rabbit immunodiffusion pattern with a specific reference band (K) in comparison with an identity (K₁) line. Wells: 1 and 4, MAb KT4; 2 and 3, MAb CA1; 5 and 6, *P. anomala* UCSC 25F yeast killer toxin; 0, rabbit antiserum.

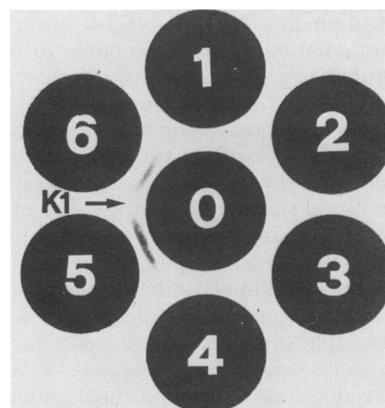


FIG. 3. Rabbit immunodiffusion pattern after absorption of MAb KT4 with *P. anomala* UCSC 25F yeast killer toxin. The disappearance of the identity line (K₁) is evident. Wells: 1 and 4, MAb CA1; 2 and 3, MAb KT4 absorbed with *P. anomala* UCSC 25F yeast killer toxin; 5 and 6, MAb KT4; 0, rabbit antiserum.

cipitin band in the system constituted from the rabbit antiserum (Fig. 3).

The evaluation of the killer activity of the serum of the rabbit immunized with the hybridoma cells secreting MAb KT4 against the yeast killer toxin of *P. anomala* UCSC 25F showed, at least in this system, an inhibitory effect on *C. albicans* CDC B385 cells (Fig. 4).

The anti-Ids produced in this study may have some promise, as they seem to include at least some fractions that display binding characteristics similar to those of the original antigen used for immunization (yeast killer toxin).

The precipitin band produced from the concentrated rabbit antiserum with the MAb KT4 was thick, sharp, and homologous to that (K) of the reference system. This observation suggested that the major part, if not all, of the reacting fractions of the rabbit antiserum was highly specific for unique variable-region determinants of the MAb (Fig. 2). This expectation was presumably confirmed by the disappearance of the K1 precipitin band after absorption of the MAb KT4 with the yeast killer toxin (Fig. 3).

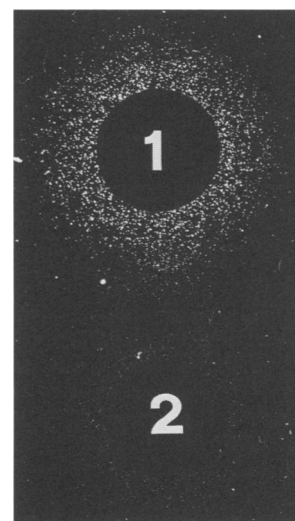


FIG. 4. Toxinlike effect of rabbit anti-Ids on *C. albicans* cells in minimal agarose medium. Wells: 1, concentrated (5×) normal rabbit serum; 2, concentrated (5×) immunized rabbit antiserum.

The observation that the MAb KT4, absorbed with the killer toxin (original antigen), was unable to react with the rabbit antiserum suggests that the antigen blocked the binding site of the MAb. Rabbit antiserum should be regarded, therefore, as anti-Ids possessing subpopulations similar or adjacent to the antigen-binding site of the MAb.

Some studies (17–19) on cell receptors have suggested that, because of the enormous heterogeneity in the antibody population, it is presumable that among all the different antibodies that are raised as a result of the immunization with the receptor-combining antigen, a few or most of the antibodies could recognize the antigen in a way that is similar to how a physiological cell receptor recognizes such an antigen. In our study, the combining site of MAb KT4 could be considered as a receptorlike antibody showing structural features in common with the toxin-binding part of the receptor. It has been apparently confirmed by the observation that at least some of the anti-Ids against the MAb KT4 to killer toxin inhibited *C. albicans* cells. Other natural antibodies (rabbit normal serum), as well as another immune rabbit antiserum unrelated to yeast killer toxin, did not display such an effect (Fig. 4).

Because the action of the anti-Ids on *C. albicans* cells differed by several magnitudes in comparison with that of the yeast killer toxin, it is difficult to evaluate the interactions in quantitative terms. It could be explained by the assumption that only a very few anti-Ids strictly met the structural requirements for the interaction with the *C. albicans* cell receptors.

It should be expected, moreover, that different animals and, presumably, immunization schedules may produce antisera that differ either in qualitative or quantitative terms. To our knowledge, this is the first report of anti-Ids mimicking the action of a microbial toxin.

At our institute, current studies are aimed to produce monoclonal anti-Ids directed against the idiotypic determinant of a monoclonal anti-yeast killer toxin antibody. Such a MAb, because of its unlimited availability and absolute reproducibility, should represent a powerful reagent for exploring the structure-function relationships between a cell-surface receptor and yeast killer toxin.

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